NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

PYRIDINE

(CAS NO. 110-86-1)

IN F344/N RATS, WISTAR RATS, AND B6C3F$_1$ MICE

(DRINKING WATER STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

March 2000

NTP TR 470

NIH Publication No. 00-3960

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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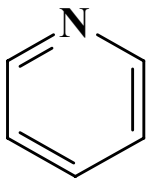
CONTENTS

ABSTRACT



PYRIDINE

CAS No. 110-86-1

Chemical Formula: C_5H_5N Molecular Weight: 79.10

Synonyms: Azabenzene, azine

Pyridine is used as a denaturant in alcohol and antifreeze mixtures, as a solvent for paint, rubber, and polycarbonate resins, and as an intermediate in the manufacture of insecticides, herbicides, and fungicides. It is used in the production of piperidine, an intermediate in the manufacture of rubber and mepiquat chloride, and as an intermediate and solvent in the preparation of vitamins and drugs, dyes, textile water repellants, and flavoring agents in food. Pyridine was nominated for study because of its large production volume and its use in a variety of food, medical, and industrial products. Male and female F344/N rats, male Wistar rats, and male and female $B6C3F_1$ mice were exposed to pyridine (approximately 99% pure) in drinking water for 13 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, L5178Y mouse lymphoma cells, cultured Chinese hamster ovary cells, *Drosophila melanogaster*, and mouse bone marrow cells.

13-WEEK STUDY IN F344/N RATS

Groups of 10 male and 10 female F344/N rats were exposed to pyridine in drinking water at concentrations of 0, 50, 100, 250, 500, or 1,000 ppm (equivalent to average daily doses of 5, 10, 25, 55, or 90 mg pyridine/kg body weight). Two females exposed to 1,000 ppm died during week 1. Final mean body weights of 1,000 ppm males and females and 500 ppm females were significantly less than controls. Water consumption by female rats exposed to 1,000 ppm was less than that by controls. At study termination, evidence of anemia persisted in the 500 and 1,000 ppm males and all exposed groups of females. There was evidence of hepatocellular injury and/or altered hepatic function demonstrated by increased serum alanine aminotransferase and sorbitol dehydrogenase activities and bile acid concentrations in 500 and 1,000 ppm rats. The estrous cycle length of 1,000 ppm females was significantly longer than that of the controls. Liver weights of males and females exposed to 250 ppm or greater were significantly greater than controls. In the liver, the incidences of centrilobular degeneration, hypertrophy, chronic inflammation, and pigmentation were generally increased in 500 and 1,000 ppm males and females relative to controls. In the kidney, the incidences of granular casts and hyaline degeneration (hyaline droplets) were significantly increased in 1,000 ppm males and slightly increased in 500 ppm males; these lesions are consistent with α2u-globulin nephropathy. Additionally, there were increased incidences and/or severities of protein casts, chronic

inflammation, mineralization, and regeneration primarily in 500 and 1,000 ppm males.

13-WEEK STUDY IN MALE WISTAR RATS

Groups of 10 male Wistar rats were exposed to pyridine in drinking water at concentrations of 0, 50, 100, 250, 500, or 1,000 ppm (equivalent to average daily doses of 5, 10, 30, 60, or 100 mg/kg). One male rat exposed to 500 ppm died during week 1. Final mean body weights of rats exposed to 250, 500, or 1,000 ppm were significantly less than those of the controls. Water consumption by rats exposed to 1,000 ppm was lower than that by controls. There was evidence of hepatocellular injury and/or altered hepatic function in the 500 and 1,000 ppm groups, similar to that observed in the 13-week study in F344/N rats. Incidences of centrilobular degeneration, hypertrophy, chronic inflammation, and pigmentation in the liver of rats exposed to 500 or 1,000 ppm were significantly increased relative to controls.

13-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F$_1$ mice were exposed to pyridine in drinking water at concentrations of 0, 50, 100, 250, 500, or 1,000 ppm (equivalent to average daily doses of 10, 20, 50, 85, or 160 mg/kg for males and 10, 20, 60, 100, or 190 mg/kg for females). One female mouse exposed to 250 ppm died during week 2. Final mean body weights of female mice exposed to 1,000 ppm were significantly less than those of controls. Water consumption by exposed female mice was lower than that by controls at week 1 but generally slightly higher than controls at week 13. Sperm motility in exposed male mice was significantly decreased relative to controls. Liver weights were significantly increased relative to controls in males exposed to 100 ppm or greater and in 250 and 500 ppm females. No chemical-related lesions were observed in male or female mice.

2-YEAR STUDY IN F344/N RATS

Groups of 50 male and 50 female F344/N rats were exposed to pyridine in drinking water at concentrations of 0, 100, 200, or 400 ppm (equivalent to average daily doses of 7, 14, or 33 mg/kg) for 104 (males) or 105 (females) weeks.

Survival, Body Weights, and Water Consumption

Survival of exposed males and females was similar to that of controls. Mean body weights of 400 ppm males and females were generally less than those of the controls throughout the study, and those of 200 ppm males and females were less during the second year of the study. Water consumption by males and females exposed to 200 or 400 ppm was generally greater than that by controls.

Pathology Findings

Incidences of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) in male rats exposed to 400 ppm were significantly increased compared to controls and exceeded the historical control ranges. The findings from an extended evaluation (step section) of the kidneys did not reveal additional carcinomas, but additional adenomas were observed in each group of males. In the standard evaluation, an increased incidence of renal tubule hyperplasia was observed in 400 ppm males compared to controls. Incidences of mononuclear cell leukemia in female rats were significantly increased in the 200 and 400 ppm groups, and the incidence in the 400 ppm group exceeded the historical control range.

Exposure concentration-related nonneoplastic liver lesions were observed in males and females, and the incidences were generally increased in groups exposed to 400 ppm. These included centrilobular cytomegaly, cytoplasmic vacuolization, periportal fibrosis, fibrosis, centrilobular degeneration and necrosis, and pigmentation. Bile duct hyperplasia occurred more often in exposed females than in controls.

2-YEAR STUDY IN MALE WISTAR RATS

Groups of 50 male Wistar rats were exposed to pyridine in drinking water at concentrations of 0, 100, 200, or 400 ppm (equivalent to average daily doses of 8, 17, or 36 mg/kg) for 104 weeks.

Survival, Body Weights, and Water Consumption

Survival of rats exposed to 200 or 400 ppm was significantly less than that of the controls. Mean body weights of rats exposed to 100, 200, or 400 ppm were significantly less than controls. Water consumption was similar by control and exposed rats.

Pathology Findings

The incidence of testicular interstitial cell adenoma in rats exposed to 400 ppm was significantly increased compared to controls. Incidences of interstitial cell hyperplasia were observed in control and exposed groups and were slightly, but not significantly, increased in rats exposed to 200 or 400 ppm.

Severity of nephropathy was marked in all groups, and additional evidence of kidney disease, including mineralization in the glandular stomach, parathyroid gland hyperplasia, and fibrous osteodystrophy, was observed in 100 and 200 ppm rats. The incidences of hepatic centrilobular degeneration and necrosis, fibrosis, periportal fibrosis, and/or pigmentation were increased in one or more exposed groups.

2-YEAR STUDY IN MICE

Groups of 50 male B6C3F$_1$ mice were exposed to pyridine in drinking water at concentrations of 0, 250, 500, or 1,000 ppm (equivalent to average daily doses of 35, 65, or 110 mg/kg) for 104 weeks, and groups of 50 female B6C3F$_1$ mice were exposed to pyridine in drinking water at concentrations of 0, 125, 250, or 500 ppm (equivalent to average daily doses of 15, 35, or 70 mg/kg) for 105 weeks.

Survival, Body Weights, and Water Consumption

Survival of exposed males and females was similar to that of the controls. Mean body weights of 250 and 500 ppm females were less than controls. Water consumption by males exposed to 250 or 500 ppm was generally greater than that by controls during the last year of the study; male mice exposed to 1,000 ppm consumed less water than controls throughout the study. Water consumption by exposed females was generally lower than that by controls during the first year of the study, but greater than controls during the second year.

Pathology Findings

Hepatocellular neoplasms, including hepatoblastomas, in exposed male and female mice were clearly related to pyridine exposure. Additionally, many mice had multiple hepatocellular neoplasms. The incidences of hepatocellular neoplasms in exposed males and females generally exceeded the historical control ranges for drinking water studies. Neoplasms from control mice, 1,000 ppm males, and 500 ppm females were negative when stained for p53 protein.

GENETIC TOXICOLOGY

Pyridine was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 or in L5178Y mouse lymphoma cells, with or without S9 metabolic activation, and it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells, with or without S9. Pyridine was tested for induction of sex-linked recessive lethal mutations in adult male *Drosophila melanogaster*, and mixed results were obtained. In one experiment, administration by injection gave negative results, but feeding produced an equivocal response. A second experiment generated negative results by injection and feeding. A third experiment showed significant increases in sex-linked recessive lethal mutations in flies treated with pyridine by injection but not by feeding. Overall, results of the sex-linked recessive lethal mutations test in *Drosophila melanogaster* were considered negative by feeding and equivocal by injection. Results of a single reciprocal translocation test in male *Drosophila melanogaster* were negative. No induction of chromosomal aberrations or micronuclei was noted in bone marrow cells of male mice administered pyridine via intraperitoneal injection.

CONCLUSIONS

Under the conditions of these 2-year drinking water studies, there was *some evidence of carcinogenic activity** of pyridine in male F344/N rats based on increased incidences of renal tubule neoplasms. There was *equivocal evidence of carcinogenic activity* of pyridine in female F344/N rats based on increased inci-dences of mononuclear cell leukemia. There was *equivocal evidence of carcinogenic activity* in male Wistar rats based on an increased incidence of interstitial cell adenoma of the testis. There was *clear evidence of carcinogenic activity* of pyridine in male and female $B6C3F_1$ mice based on increased incidences of malignant hepatocellular neoplasms.

In F344/N rats, exposure to pyridine resulted in increased incidences of centrilobular cytomegaly and degeneration, cytoplasmic vacuolization, and pigmentation in the liver of males and females; periportal fibrosis, fibrosis, and centrilobular necrosis in the liver of males; and bile duct hyperplasia in females. In male Wistar rats, pyridine exposure resulted in increased incidences of centrilobular degeneration and necrosis, fibrosis, periportal fibrosis, and pigmentation in the liver, and, secondary to kidney disease, mineralization in the glandular stomach and parathyroid gland hyperplasia.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 15.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Pyridine

	Male F344/N Rats	Female F344/N Rats	Male Wistar Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in drinking water	0, 100, 200, or 400 ppm	0, 100, 200, or 400 ppm	0, 100, 200, or 400 ppm	0, 250, 500, or 1,000 ppm	0, 125, 250, or 500 ppm
Body weights	200 and 400 ppm groups less than control group	200 and 400 ppm groups less than control group	Exposed groups less than control group	Exposed groups similar to control group	Exposed groups less than control group
Survival rates	25/50, 20/50, 25/50, 16/50	32/50, 37/50, 29/50, 26/50	22/50, 14/50, 11/50, 7/50	35/50, 28/50, 35/49, 35/50	32/50, 30/50, 22/50, 29/50
Nonneoplastic effects	Liver: centrilobular cytomegaly (0/50, 4/49, 8/50, 6/50); cytoplasmic vacuolization (4/50, 6/49, 13/50, 17/50); periportal fibrosis (0/50, 0/49, 2/50, 29/50); fibrosis (1/50, 1/49, 1/50, 10/50); centrilobular degeneration (1/50, 3/49, 2/50, 8/50); centrilobular necrosis (0/50, 3/49, 0/50, 5/50); pigmentation (4/50, 11/49, 20/50, 25/50)	Liver: centrilobular cytomegaly (0/50, 1/50, 4/50, 20/50); cytoplasmic vacuolization (10/50, 7/50, 9/50, 18/50); centrilobular degeneration (1/50, 2/50, 2/50, 7/50); bile duct hyperplasia (20/50, 29/50, 34/50, 29/50); pigmentation (6/50, 2/50, 6/50, 17/50)	Liver: centrilobular degeneration (1/50, 15/50, 25/50, 33/50); centrilobular necrosis (5/50, 6/50, 4/50, 23/50); fibrosis (1/50, 5/50, 26/50, 31/50); periportal fibrosis (0/50, 0/50, 5/50, 7/50); pigmentation (6/50, 15/50, 34/50, 42/50) Glandular Stomach: mineralization (8/49, 25/50, 16/48, 6/48) Parathyroid Gland: hyperplasia (16/48, 32/47, 29/48, 12/47)	None	None
Neoplastic effects	Kidney: renal tubule adenoma (standard evaluation - 1/50, 0/48, 2/50, 6/49; standard and extended evaluations combined- 2/50, 3/48, 6/50, 10/49); renal tubule adenoma or carcinoma (standard evaluation - 1/50, 1/48, 2/50, 6/49; standard and extended evaluations combined- 2/50, 4/48, 6/50, 10/49)	None	None	Liver: hepatocellular adenoma (29/50, 40/50, 34/49, 39/50); hepatocellular carcinoma (15/50, 35/50, 41/49, 40/50); hepatoblastoma (2/50, 18/50, 22/49, 15/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (38/50, 47/50, 46/49, 47/50)	Liver: hepatocellular adenoma (37/49, 39/50, 43/50, 34/50); hepatocellular carcinoma (13/49, 23/50, 33/50, 41/50); hepatoblastoma (1/49, 2/50, 9/50, 16/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (41/49, 42/50, 45/50, 44/50)
Uncertain findings	None	Mononuclear cell leukemia: (12/50, 16/50, 22/50, 23/50)	Testis: interstitial cell adenoma (5/50, 6/49, 4/49, 12/50)	None	None
Level of evidence of carcinogenic activity	Some evidence	Equivocal evidence	Equivocal evidence	Clear evidence	Clear evidence

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Pyridine

Genetic toxicology	
Salmonella typhimurium gene mutations:	Negative in strains TA98, TA100, TA1535, and TA1537, with and without S9
Mouse lymphoma gene mutations:	Negative with and without S9
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Chromosomal aberrations	Negative with and without S9
Cultured Chinese hamster ovary cells *in vitro*:	Negative
Mouse bone marrow *in vivo*:	Equivocal by injection; negative by feeding
Sex-linked recessive lethal mutations	
Drosophila melanogaster:	Negative
Reciprocal translocations	
Drosophila melanogaster:	Negative
Micronucleated erythrocytes	
Mouse bone marrow *in vivo*:	

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on pyridine on 10 December 1997 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 10 December 1997, the draft Technical Report on the toxicology and carcinogenesis studies of pyridine received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of pyridine by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on any survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *some evidence of carcinogenic activity* in male F344/N rats, *equivocal evidence of carcinogenic activity* in female F344/N rats and male Wistar rats, and *clear evidence of carcinogenic activity* in male and female B6C3F_1 mice.

Dr. Cullen, a principal reviewer, agreed with the proposed conclusions. He noted the large amount of inflammation in mouse livers and asked whether they had been screened for the possible presence of *Helicobacter hepaticus* infection. Dr. J.R. Hailey, NIEHS, said there was no frozen tissue available to perform PCR-based assays for identification of *H. hepaticus*. However, the liver lesions observed were not consistent with those typically associated with *H. hepaticus* infection.

Dr. Fischer, the second principal reviewer, agreed with the conclusions. She said the discussion should include comments on increased incidences of metastatic neoplasms in mice compared to rats. Dr. Dunnick agreed. Dr. Fischer expressed concern that the Wistar rats exposed to 400 ppm did not live long enough to produce neoplasms, and, thus, this experiment was not informative.

Dr. Bus, the third principal reviewer, did not agree with the proposed conclusions for female rats and mice and for male Wistar rats. He said the proposed conclusion of equivocal evidence in female rats was not warranted based on the lack of dose response, incidence values that only slightly exceeded recent NTP historical control values, and excessive body weight depressions that confound interpretation of chemical-associated neoplasms. Dr. Dunnick responded that by definition, the increases in the incidences of mononuclear cell leukemia were uncertain findings. With regard to male Wistar rats, Dr. Bus stated that the severe toxicity associated with markedly decreased survival and effects on body weight gain, especially at 200 and 400 ppm, compromised interpretation of the increased incidence of testicular adenomas in the 400 ppm group. Finally, he thought it difficult to understand a conclusion of clear evidence in female mice in view of the profound body weight loss over the last 25 weeks of the study, and though there was an exposure-related increase in the incidences of malignant liver neoplasms, liver adenomas and total neoplasms were not altered. Dr. Dunnick said the level of clear evidence was justified by the large exposure-related increased incidences of malignant neoplasms. The body weight loss was due in part to the development of liver neoplasms. Dr. J.K. Haseman, NIEHS, noted that while the incidence of liver neoplasms in control female mice may have been one of the highest seen in the NTP, almost all neoplasms were adenomas. On the other hand, almost every exposed animal that lived one year or longer developed a liver neoplasm, often multiple neoplasms, and often carcinomas or hepatoblastomas, with many neoplasms metastasizing to the lung, constituting one of the strongest carcinogenic effects ever seen at this site in his experience. Dr. Bus said this changed his perspective on the neoplasms in female mice.

Further discussion of whether hepatoblastomas should be viewed and weighed separately from hepatocellular carcinomas ensued. Dr. Hailey thought they should be viewed as part of a natural progression and that with chemicals having neoplasm promoter activity there is almost always an associated increase in hepatoblastomas. There was discussion about the appropriateness in general of combining benign and malignant neoplasms. Dr. J. Russo argued that combining can be misleading. Dr. Hailey commented that with some neoplasm types combining might be controversial but with the liver (mice) and the kidney (rats), the sites at issue here, there is a spectrum of lesions

from foci or hyperplasia to adenoma to carcinoma that represents a morphological and biological continuum, and combining seems appropriate. Dr. Bailer said that, based on the data in the report, he would have considered clear evidence as the proposed conclusion for male rats. Dr. Bucher observed that NTP is using its combined experience to delineate between some evidence and clear evidence based on its historical perspective.

Dr. Bus moved that the Technical Report on pyridine be accepted with the revisions discussed and the conclusions as written for male F344/N rats, *some evidence of carcinogenic activity*, and for male and female B6C3F_1 mice, *clear evidence of carcinogenic activity*. He moved that the conclusions for female F344/N rats and male Wistar rats be changed from *equivocal evidence of carcinogenic activity* to *inadequate study of carcinogenic activity*. Dr. Cullen seconded the motion. Dr. Haseman said that *inadequate study* is a category of evidence generally used only when there is some major flaw that makes the study uninterpretable. Dr. Bailer moved to amend the motion to keep the level of evidence for female F344/N rats and male Wistar rats as originally proposed, *equivocal evidence of carcinogenic activity*. Dr. Cullen seconded the amendment, which was accepted by six yes votes to one no vote (Dr. Bus). Dr. Bus's motion as amended by Dr. Bailer was accepted unanimously with seven votes.